

APPENDIX A

Claim	Support Includes
1. A process for preparing a vascular endothelial growth factor (VEGF) dimer comprising:	Claims 1 and 35 of parent application 09/575,199 Paragraphs [0009], [0017], and [0028] from the original specification of current application 10/749,703.
providing transformed host bacterial cells,	Claims 35 and 43 of parent application 09/575,199 Paragraphs [0029], [0030], and [0085] from the original specification of current application 10/749,703
wherein the transformed host bacterial cells comprise an exogenous nucleic acid encoding an amino acid sequence of a VEGF monomer operably linked to a promoter,	Claims 35 and 43 of parent application 09/575,199 Paragraphs [0029] and [0133] from the original specification of current application 10/749,703
wherein the amino acid sequence has at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1	Claims 1, 14, 28, 35, and 54 of parent application 09/575,199 Paragraph [0028] from the original specification of current application 10/749,703
and wherein the amino acid sequence is extended by a Met-(AA) _n - sequence at the amino terminus (N-terminus),	Claims 45, 54, and 65 of parent application 09/575,199 Paragraphs [0029] and [0033] from the original specification of current application 10/749,703
wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids,	Claims 45, 54, and 65 of parent application 09/575,199 Paragraphs [0029] and [0033] from the original specification of current application 10/749,703
where at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of retarding proteolytic	Claims 45, 54, and 65 of parent application 09/575,199 Paragraphs [0029] and [0033] from the original specification of current application 10/749,703

Claim	Support Includes
degradation of the mature N-terminus of the VEGF dimer by the bacterial host cell,	10/749,703
and the amino acid sequence retains a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116);	Claims 1, 14, 28, 35, and 54 of parent application 09/575,199 Paragraphs [0009], [0011], and [0013] from the original specification of current application 10/749,703
culturing said host cells under conditions suitable for expression of said VEGF monomer, whereby a first VEGF monomer and a second VEGF monomer are produced;	Claim 35 and 54 of parent application 09/575,199 Paragraphs [0024] and [0030] from the original specification of current application 10/749,703
forming the VEGF dimer from the first and second VEGF monomers;	Claims 1, 14, 28, and 54 of parent application 09/575,199 Paragraph [0028] from the original specification of current application 10/749,703
and recovering said VEGF dimer.	Claims 35 and 54 of parent application 09/575,199 Paragraphs [0031] and [0094] from the original specification of current application 10/749,703
2. The process of claim 1, wherein n is 1.	Claims 46 and 55 of parent application 09/575,199 Paragraph [0027] from the original specification of current application 10/749,703
3. The process of claim 2, wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.	Claims 47 and 56 of parent application 09/575,199 Paragraph [0146] from the original specification of current application 10/749,703
4. The process of claim 3, wherein AA represents a lysine (Lys) residue.	Claims 48 and 57 of parent application 09/575,199 Paragraph [0146] from the original

Claim	Support Includes
5. The process of claim 1, further comprising the step of purifying said VEGF dimers.	specification of current application 10/749,703 Claims 42 and 49 of parent application 09/575,199 Paragraph [0093] from the original specification of current application 10/749,703
6. The process of claim 5, further comprising the removal of the N-terminal Met(AA) _n - sequence following at least partial purification.	Claims 50 and 59 of parent application 09/575,199 Paragraphs [0077] and [0094] from the original specification of current application 10/749,703
7. The process of claim 6, wherein removal is performed by enzymatic digestion.	Claims 51 and 60 of parent application 09/575,199 Paragraphs [0077] and [0094] from the original specification of current application 10/749,703
8. The process of claim 7, wherein diaminopeptidase is used to perform the enzymatic digestion.	Claims 51 and 60 of parent application 09/575,199 Paragraph [0094] from the original specification of current application 10/749,703
9. The process of claim 1, wherein at least about 95% of said VEGF dimers are devoid of an N-terminal methionine residue.	Paragraph [0140] from the original specification of current application 10/749,703
10. The process of claim 1, further comprising the step of refolding said VEGF dimers.	Claims 52, 61, 62, and 63 of parent application 09/575,199 Paragraphs [0093] and [0155] from the original specification of current application 10/749,703
11. The process of claim 10, wherein refolding is performed in a refolding buffer comprising cysteine and cystine in amounts and in	Claims 53 and 64 of parent application 09/575,199 Paragraphs [0093] and [0155] from the

Claim	Support Includes
a ratio to each other sufficient to produce the desired mixture of VEGF dimers.	original specification of current application 10/749,703
12. A process for producing a vascular endothelial growth factor (VEGF) dimer composed of two VEGF monomers,	Claims 1, 14, 28, and 54 of parent application 09/575,199 Paragraph [0028] from the original specification of current application 10/749,703
in which each monomer comprises amino acids 11 to 116 of SEQ ID NO: 1,	Claims 1, 14, 28, 35, and 54 of parent application 09/575,199 Paragraphs [0010], [0011], and [0018] from the original specification of current application 10/749,703
or comprises an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1,	Claims 1, 14, 28, 35, and 54 of parent application 09/575,199 Paragraph [0028] from the original specification of current application 10/749,703
and retaining a cysteine (Cys) at a position corresponding to position 116 of SEQ ID NO: 1 (Cys-116),	Claims 1, 14, 28, 35, and 54 of parent application 09/575,199 Paragraphs [0009], [0011], and [0013] from the original specification of current application 10/749,703
where Cys-116 of each monomer is disulfide bonded to an additional extraneous Cys, comprising the steps of:	Claims 1, 14, 28, 35, and 54 of parent application 09/575,199 Paragraph [0028] from the original specification of current application 10/749,703
providing transformed bacterial host cells comprising a species of exogenous nucleic acid encoding a promoter operably linked to a polypeptide of SEQ ID NO: 1 extended by a Met-(AA) _n sequence at the amino terminus (N-terminus),	Claims 45, 54, and 65 of parent application 09/575,199 Paragraphs [0029] and [0033] from the original specification of current application 10/749,703
wherein Met stands for methionine, n is	Claims 45, 54, and 65 of parent application 09/575,199

Claim	Support Includes
1-7, and AA represents identical or different amino acids,	Paragraphs [0029] and [0033] from the original specification of current application 10/749,703
wherein at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of blocking the proteolytic degradation of the mature N-terminus of the VEGF polypeptides by the bacterial host cell;	Claims 45, 54, and 65 of parent application 09/575,199 Paragraphs [0029] and [0033] from the original specification of current application 10/749,703

Claim	Support Includes
culturing said bacterial host cells under conditions suitable for expression of said exogenous nucleic acid and the synthesis of said N-terminally-extended VEGF monomers,	Claims 54 of parent application 09/575,199 Paragraphs [0024], [0029], [0030] from the original specification of current application 10/749,703
and recovering said VEGF dimer.	Claims 35 and 54 of parent application 09/575,199 Paragraphs [0031] and [0094] from the original specification of current application 10/749,703
13. The process of claim 12, wherein n is 1.	Claims 46 and 55 of parent application 09/575,199 Paragraph [0027] from the original specification of current application 10/749,703
14. The process of claim 13, wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.	Claims 47 and 56 of parent application 09/575,199 Paragraph [0146] from the original specification of current application 10/749,703
15. The process of claim 14, wherein AA represents a lysine (Lys) residue.	Claims 48 and 57 of parent application 09/575,199 Paragraph [0146] from the original specification of current application 10/749,703
16. The process of claim 12, further comprising the step of purifying said VEGF dimer.	Claims 42 and 49 of parent application 09/575,199 Paragraph [0093] from the original specification of current application 10/749,703
17. The process of claim 16, further comprising the removal of the N-terminal Met(AA) _n sequence following at least partial purification.	Claims 50 and 59 of parent application 09/575,199 Paragraphs [0077] and [0094] from the original specification of current application 10/749,703
18. The process of claim 17, wherein	Claims 51 and 60 of parent application 09/575,199

Claim	Support Includes
removal is performed by enzymatic digestion.	Paragraphs [0077] and [0094] from the original specification of current application 10/749,703
19. The process of claim 18, wherein at least about 95% of said VEGF dimers are devoid of an N-terminal methionine residue.	Claims 51 and 60 of parent application 09/575,199 Paragraphs [0077], [0094], and [0140] from the original specification of current application 10/749,703
20. The process of claim 12, additionally comprising the step of refolding said VEGF dimer.	Claims 52, 61, 62, and 63 of parent application 09/575,199 Paragraphs [0093] and [0155] from the original specification of current application 10/749,703
21. The process of claim 14, additionally comprising the step of refolding said VEGF dimer.	Claims 52, 61, 62, and 63 of parent application 09/575,199 Paragraphs [0093] and [0155] from the original specification of current application 10/749,703
22. The process of claim 17, additionally comprising the step of refolding said VEGF dimer.	Claims 52, 61, 62, and 63 of parent application 09/575,199 Paragraphs [0093] and [0155] from the original specification of current application 10/749,703
23. The process of claim 22, wherein refolding is performed in a refolding buffer comprising cysteine and cystine.	Claims 53 and 64 of parent application 09/575,199 Paragraphs [0093] and [0155] from the original specification of current application 10/749,703
24. A process for preparing a vascular endothelial growth factor (VEGF) dimer comprising:	Claims 1 and 35 of parent application 09/575,199 Paragraphs [0009], [0017], and [0028] from the original specification of current application 10/749,703.
providing host cells,	Claims 35 and 43 of parent application 09/575,199

Claim	Support Includes
	Paragraphs [0029], [0030], and [0085] from the original specification of current application 10/749,703
wherein the host cells comprise an exogenous nucleic acid encoding an amino acid sequence of a VEGF monomer operably linked to a promoter,	Claims 35 and 43 of parent application 09/575,199 Paragraphs [0029] and [0133] from the original specification of current application 10/749,703
wherein the amino acid sequence has at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1,	Claims 1, 14, 28, 35, and 54 of parent application 09/575,199 Paragraph [0028] from the original specification of current application 10/749,703
retains a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116),	Claims 1, 14, 28, 35, and 54 of parent application 09/575,199 Paragraphs [0009], [0011], and [0013] from the original specification of current application 10/749,703
and wherein at least one monomer has an Asn-to-Glu amino acid substitution at or corresponding to position 75 of SEQ ID NO: 1;	Paragraph [0162] from the original specification of current application 10/749,703
culturing said host cells under conditions suitable for expression of said VEGF monomer, whereby a first VEGF monomer and a second VEGF monomer are produced;	Claim 35 and 54 of parent application 09/575,199 Paragraphs [0024] and [0030] from the original specification of current application 10/749,703

Claim	Support Includes
forming the VEGF dimer from the first and second VEGF monomers;	Claims 1, 14, 28, and 54 of parent application 09/575,199 Paragraph [0028] from the original specification of current application 10/749,703
and recovering said VEGF dimer.	Claims 35 and 54 of parent application 09/575,199 Paragraphs [0031] and [0094] from the original specification of current application 10/749,703
25. The process of claim 24, wherein each monomer comprises amino acids 1 to 120 of SEQ ID NO: 1.	Claims 6 and 19 of parent application 09/575,199 Paragraph [0016] from the original specification of current application 10/749,703
26. The process of claim 24, wherein monomer comprises amino acids 1 to 121 of SEQ ID NO: 1.	Claims 7, 20, 30, 37, and 40 of parent application 09/575,199 Paragraphs [0035] and [0036] from the original specification of current application 10/749,703
27. The process of claim 24, wherein at least about 95% of said VEGF dimers are devoid of an N-terminal methionine residue.	Paragraph [0140] from the original specification of current application 10/749,703
28. The process of claim 24, wherein the Cys residue corresponding to Cys-116 of SEQ ID NO:1 of each monomer is disulfide bonded to an extraneous Cys.	Claims 1, 14, 28, 35, and 54 of parent application 09/575,199 Paragraph [0028] from the original specification of current application 10/749,703
29. The process of claim 24, wherein the Cys residue corresponding to Cys-116 of SEQ ID NO:1 of the two monomers are interconnected with an interchain disulfide bond.	Claims 26, 28, and 35 of parent application 09/575,199 Paragraph [0039] and Figure 4 from the original specification of current application 10/749,703
30. The process of claim 24, wherein the Cys residue corresponding to Cys-116 of SEQ	Claims 28 and 35 of parent application 09/575,199 Paragraphs [0045], [0155], [0178], [0179],

Claim	Support Includes
ID NO:1 of one or both monomers is not reduced.	[0180] from the original specification of current application 10/749,703
31. The process of claim 24, additionally comprising the step of purifying said dimers.	Claims 42 and 49 of parent application 09/575,199 Paragraph [0093] from the original specification of current application 10/749,703
32. The process of claim 24, wherein said transformed host cells are bacterial cells.	Claims 43, 54, and 65 of parent application 09/575,199 Paragraph [0085] from the original specification of current application 10/749,703
33. The process of claim 32, wherein said bacterial cells are <i>E. coli</i> cells.	Claim 44 of parent application 09/575,199 Paragraph [0085] from the original specification of current application 10/749,703
34. The process of claim 32, wherein the exogenous nucleic acid encodes a polypeptide of SEQ ID NO: 1 extended by a Met-(AA) _n -sequence at the amino terminus (N-terminus),	Claims 45, 54, and 65 of parent application 09/575,199 Paragraphs [0029] and [0033] from the original specification of current application 10/749,703
wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids,	Claims 45, 54, and 65 of parent application 09/575,199 Paragraphs [0029] and [0033] from the original specification of current application 10/749,703
where at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of retarding proteolytic degradation of the mature N-terminus of the VEGF dimer by the bacterial host cell.	Claims 45, 54, and 65 of parent application 09/575,199 Paragraphs [0029] and [0033] from the original specification of current application 10/749,703
35. The process of claim 34, wherein n	Claims 46 and 55 of parent application 09/575,199 Paragraph [0027] from the original

Claim	Support Includes
is 1.	specification of current application 10/749,703
36. The process of claim 35, wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.	Claims 47 and 56 of parent application 09/575,199 Paragraph [0146] from the original specification of current application 10/749,703
37. The process of claim 36, wherein AA represents a lysine (Lys) residue.	Claims 48 and 57 of parent application 09/575,199 Paragraph [0146] from the original specification of current application 10/749,703
38. The process of claim 34, further comprising the step of purifying said VEGF dimers.	Claims 42 and 49 of parent application 09/575,199 Paragraph [0093] from the original specification of current application 10/749,703
39. The process of claim 38, further comprising the removal of the N-terminal Met(AA) _n - sequence following at least partial purification.	Claims 50 and 59 of parent application 09/575,199 Paragraphs [0077] and [0094] from the original specification of current application 10/749,703
40. The process of claim 39, wherein removal is performed by enzymatic digestion.	Claims 51 and 60 of parent application 09/575,199 Paragraphs [0077] and [0094] from the original specification of current application 10/749,703
41. The process of claim 32, further comprising the step of refolding said VEGF dimers.	Claims 52, 61, 62, and 63 of parent application 09/575,199 Paragraphs [0093] and [0155] from the original specification of current application 10/749,703
42. The process of claim 41, wherein	Claims 53 and 64 of parent application 09/575,199

Claim	Support Includes
refolding is performed in a refolding buffer comprising cysteine and cystine in amounts and in a ratio to each other sufficient to produce the desired mixture of VEGF dimers.	Paragraphs [0093] and [0155] from the original specification of current application 10/749,703